EXHIBIT A

08/967826 11/12/97

Class 123 Subclass 32

ISSUE CLASSIFICATION

[redacted]

6409840

UTILITY SERIAL NUMBER	PATENT DATE	PATENT NUMBER
	JUN 25 2002	6409840

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
08/967,826	11/12/97 RULE 60	426	32.000	1302	

APPLICANTS KENNETH J. MCNAUGHT, NORTH EPPING, AUSTRALIA; ERIC MOLONEY, TAMWORTH, AUSTRALIA; IAN L. BROWN, TAMWORTH, AUSTRALIA; ADRIAN TIMOTHY KNIGHT, LANE COVE, AUSTRALIA.

CONTINUING DATA********
VERIFIED THIS APPLN IS A DIV OF 08/374,645 04/27/95 PAT 5,714,600
WHICH IS A 371 OF PCT/AU93/00389 07/30/93

FOREIGN APPLICATIONS********
VERIFIED JAPAN PL 3994 07/31/92
JAPAN PL 7266 02/12/93

Foreign priority claimed 35 USC 119 conditions met	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☒ yes ☐ no ☒ yes ☐ no Verified and Acknowledged Examiner's Initials	→	AUX	4	8	2	$790.00	1451-007B

ADDRESS ~~LOWE PRICE LEBLANC & BECKER 99 CANAL CENTER PLAZA SUITE 22314 ALEXANDRIA VA 22314~~
McDERMOTT WILL & EMERY 600 13th Street N.W. Washington DC 20005

TITLE HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

U.S. DEPT. OF COMM./ PAT. & TM—PTO-436L (Rev.

3/7/02

PARTS OF APPLICATION FILED SEPARATELY — Applications Examiner

NOTICE OF ALLOWANCE MAILED: 2/26/02

Assistant Examiner

CLAIMS ALLOWED: Total Claims 5; Print Claim 1

ISSUE FEE: Amount Due $1980.00; Date Paid 5-13-02

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DAVID BRUNSMAN
PRIMARY EXAMINER
GROUP 1100
Primary Examiner
5/24/02

Label Area

PREPARED FOR ISSUE

ISSUE BATCH NUMBER

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PATENT APPLICATION

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Date Entered or Counted

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Date Received or Mailed

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	14.	FOAKL	2/8/02
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EXAMINER			
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INDEX OF CLAIMS

Claim Final	Claim Original	Date 4/1/98	7/13/98	12/16/98	1/7/99	2/22/99		
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SYMBOLS

V	Rejected
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-	(Through numerals) Canceled
+	Restricted
N	Non-elected
I	Interference
A	Appeal
O	Objected

Claim Final	Claim Original	Date						
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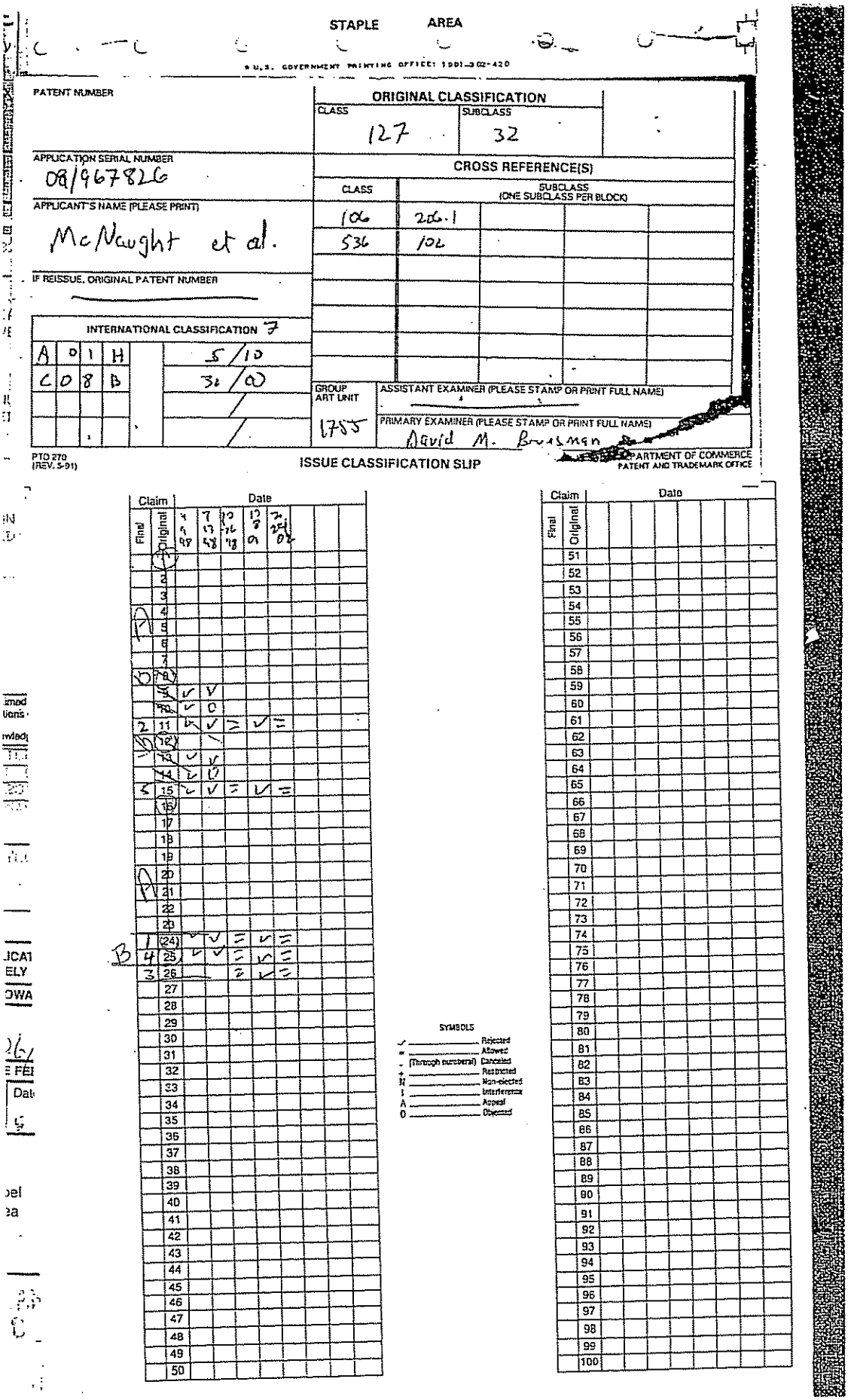

STAPLE AREA

* U.S. GOVERNMENT PRINTING OFFICE: 1991—302-420

PATENT NUMBER

APPLICATION SERIAL NUMBER: 08/967826

APPLICANT'S NAME (PLEASE PRINT): McNaught et al.

IF REISSUE, ORIGINAL PATENT NUMBER

INTERNATIONAL CLASSIFICATION 7

A	0	1	H	5/10
C	0	8	B	3/00
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ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
127	32

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
106	206.1			
536	102			

GROUP ART UNIT: 1755

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME): David M. Brunsman

PTO 270 (REV. 5-91)

ISSUE CLASSIFICATION SLIP

DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

Claim Final	Claim Original	Date 4/9/98	7/13/98	10/26/98	12/9/01	2/25/02
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	(12)		/			
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	14	✓	O			
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SYMBOLS

- ✓ ____ Rejected
- = ____ Allowed
- - (Through numeral) Canceled
- + ____ Restricted
- N ____ Non-elected
- I ____ Interference
- A ____ Appeal
- O ____ Objected

Claim Final	Claim Original	Date
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SEARCHED

Class	Sub.	Date	Exmr.
127 536 156	32 102 200.1	4/19/98	MM

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
127 536 156	32 102 200.1	4/19/98	MM

SEARCH NOTES

	Date	Exmr.
checked present USPAT STN Logs	2/24/02	MM

(RIGHT OUTSIDE)

US006409840B1

(12) **United States Patent**
McNaught et al.

(10) **Patent No.: US 6,409,840 B1**
(45) **Date of Patent: Jun. 25, 2002**

(54) **HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS**

(75) Inventors: **Kenneth J McNaught**, North Epping; **Eric Moloney**; **Ian L Brown**, both of Tamworth; **Adrian Timothy Knight**, Lane Cove, all of (AU)

(73) Assignee: **Goodman Fielder Limited**, New South Wales (AU)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 08/967,826

(22) Filed: Nov. 12, 1997

Related U.S. Application Data

(62) Division of application No. 08/374,645, filed as application No. PCT/AU93/00389 on Jul. 30, 1993, now Pat. No. 5,714,600.

(30) Foreign Application Priority Data

Jul. 31, 1992 (AU) PL3894
Feb. 12, 1993 (AU) PL7266

(51) Int. Cl.[7] A01H 5/10; C08B 30/00
(52) U.S. Cl. 127/32; 106/206.1; 536/102
(58) Field of Search 127/32; 536/102; 106/206.1

(56) References Cited

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5,714,600 A * 2/1998 McNaught et al. 536/102

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AU 45616/89 5/1990
AU PL 0537 12/1992
EP 0 118 240 9/1984

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Fornal et al., "Chemical Characteristics and Physico–chemical Properties of the Extruded Mistures of Cereal Starches", Starch/Starke 39(1987) no month avail. Nr. 3, p. 75–78.
Cluskey et al., "Fractionation and Characterization of Dent Corn and Amylomaize Starch Granules", Peoria—Starch/Starke 32 (1980) no month aval. Nr. 4. S. 105–109.
Cereal Chemistry—vol. 52, No. 6, Nov. (Dec. 1975.).
Pomeranz et al., "Corn Hardness Determination", Cereal Chemistry 61(2):174–150, vol. 61, No. 5, (1984.) no month avail.
Szczodrak et al., "Starch and Enzyme–Resistant Starch from High–Amylose Barley", pp. 589–596, (1991.) no moth avail.

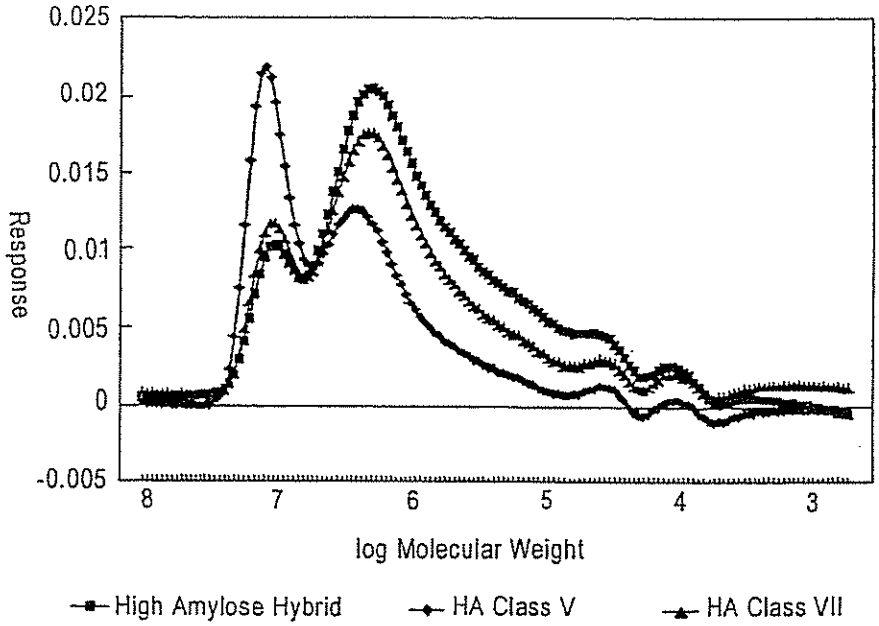
* cited by examiner

Primary Examiner—David Brunsman
(74) *Attorney, Agent, or Firm*—McDermott, Will & Emery

(57) **ABSTRACT**

Starch, particularly maize starch, having an amylose content of more than 80% w/w, including physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof. Also, disclosed are hybrid maize seeds capable of producing a starch having an amylose content of more than 80%. Also disclosed are starch fractions of enhanced dietary fiber and/or resistant starch content.

5 Claims, 4 Drawing Sheets


0.025
0.02
0.015
0.01
0.005
0
-0.005
Response
8
7
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3
log Molecular Weight
High Amylose Hybrid
HA Class V
HA Class VII

FIG. 1

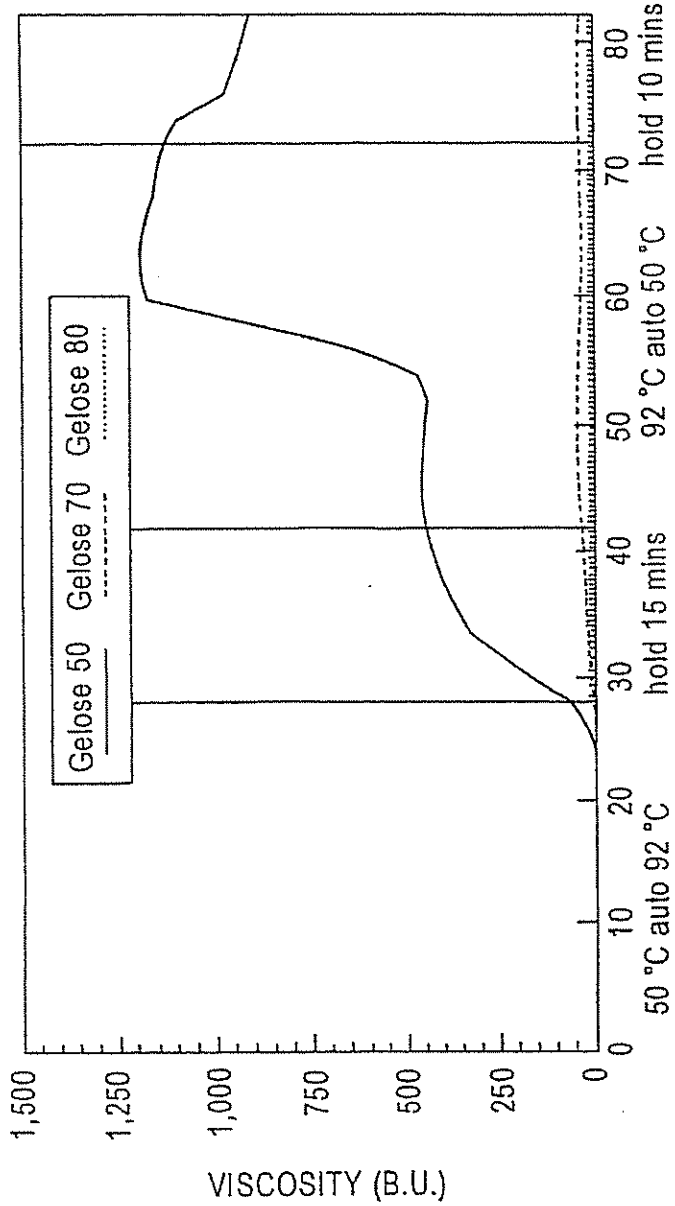
Gelose 50
Gelose 70
Gelose 80
VISCOSITY (B.U.)
1,500
1,250
1,000
750
500
250
0
0
10
20
30
40
50
60
70
80
50 °C auto 92 °C
hold 15 mins
92 °C auto 50 °C
hold 10 mins
TIME (minutes)

FIG. 2

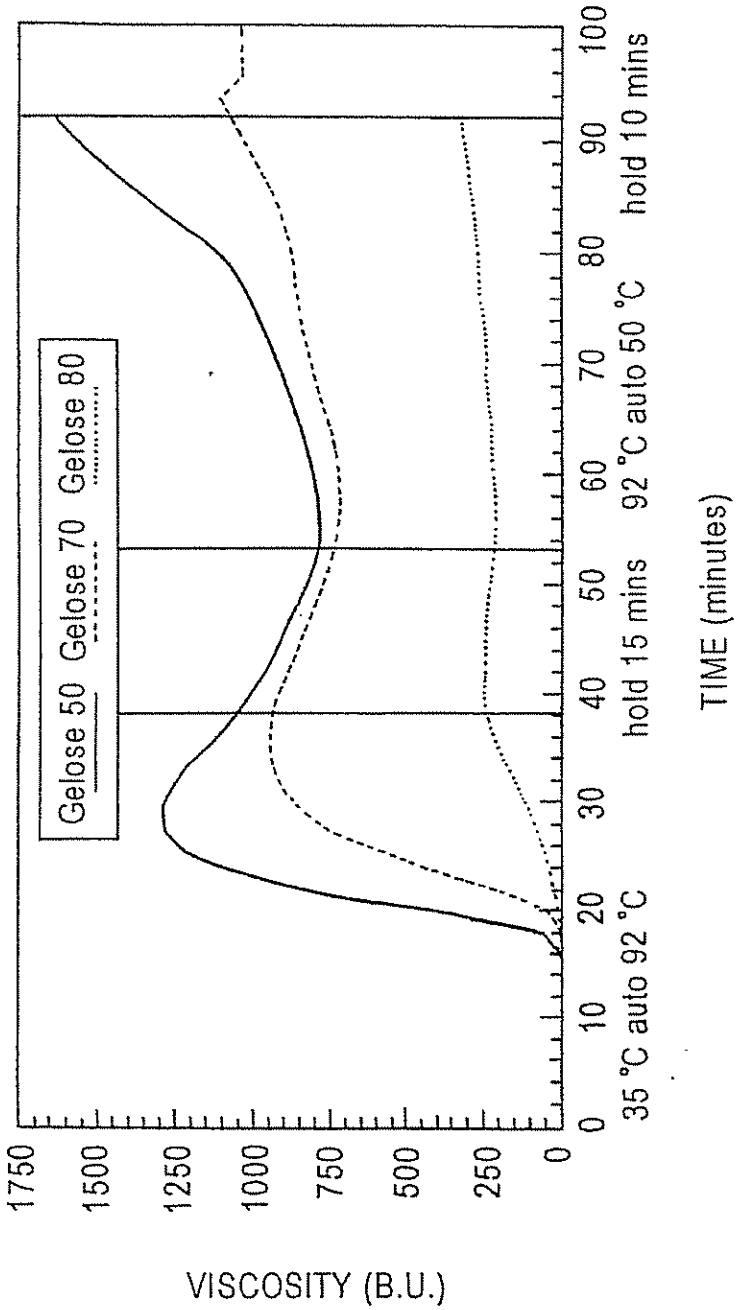
Gelose 50
Gelose 70
Gelose 80
VISCOSITY (B.U.)
1750
1500
1250
1000
750
500
250
0
0
10
20
30
40
50
60
70
80
90
100
35 °C auto 92 °C
hold 15 mins
92 °C auto 50 °C
hold 10 mins
TIME (minutes)

FIG. 3

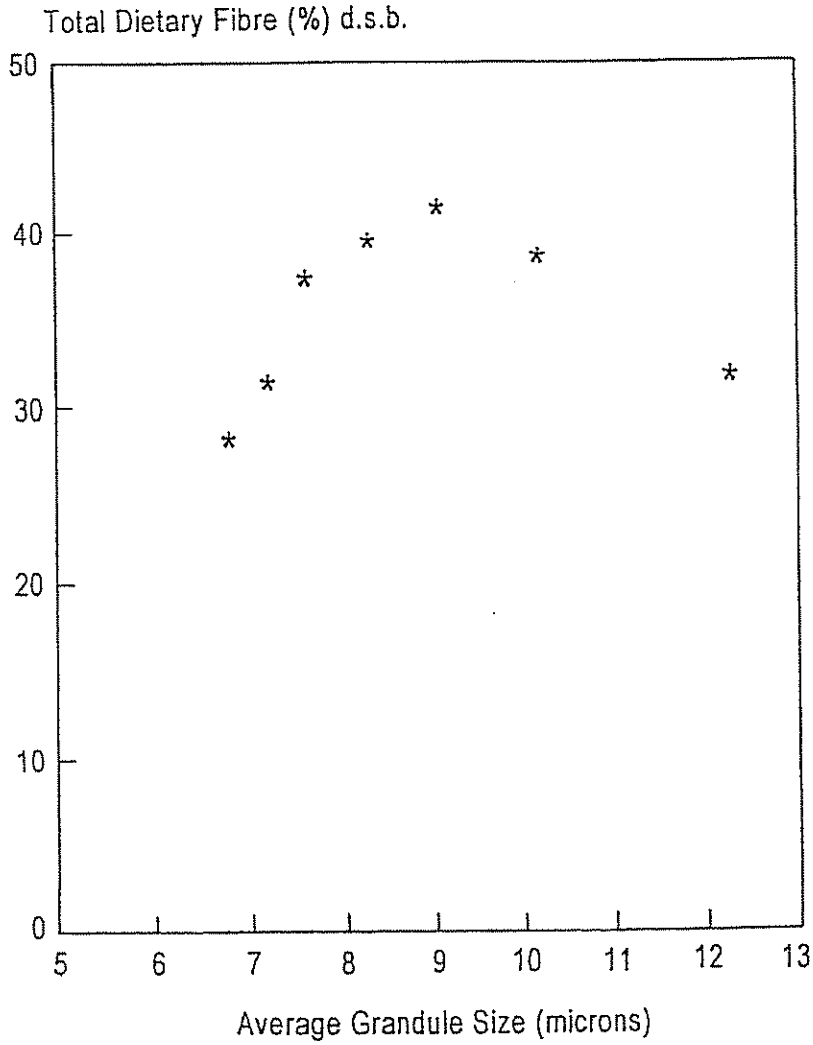
Total Dietary Fibre (%) d.s.b.
50
40
30
20
10
0
5
6
7
8
9
10
11
12
13
Average Grandule Size (microns)

FIG. 4

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

This application is a division of application Ser. No. 08/374,645 filed Apr. 27, 1995 now U.S. Pat. No. 5,714,600, which is a 371 of PCT/AU93/00389 filed Jul. 30, 1993.

TECHNICAL FIELD

This invention relates to high amylose content starch, in particular to a maize starch having an amylose content of more than 80% w/w. The invention further relates to single, double and multiple cross maize hybrids, particularly to a maize single cross F1 hybrid, capable of producing grain having such a high amylose content and to this grain.

The invention still further relates to fractions of high amylose starch that are enriched in dietary fibre and resistant starch content whilst claiming a high amylose content.

BACKGROUND ART

Most common starches contain approximately 25% amylose and 75% amylopectin. Amylose is a linear glucose polymer fraction, whilst amylopectin is a branched glucose polymer fraction.

In the prior art, it has been recognized that currently available commercial starch having an elevated amylose content would impart certain desirable properties to various compositions including films, foods and industrial products. Accordingly, attempts have been made in the prior art to produce high amylose content maize. This is exemplified in AU-A-45616/89 wherein a maize seed deposited as ATCC No. 40499 is disclosed as capable of yielding a starch having an amylose content of up to 72%.

Typically, however, a commercial starch having an amylose content of 55–65% would be regarded in the art as having a high amylose content.

The present inventors whilst recognizing the utility of the commercially available so-called high amylose starches, have sought to produce a maize having a still higher amylose content.

DISCLOSURE OF INVENTION

In the course of a breeding program, a single cross F1 hybrid maize seed was produced, which carried the ae amylose extender gene. This seed was found to be capable of producing grain, in which the amylose content of the starch derived therefrom was in excess of 80%.

Accordingly, in a first aspect, this invention consists in a hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

In a second aspect, this invention further consists in a maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

In a third aspect, this invention still further consists in compositions including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

In a fourth aspect, this invention still further consists in a process for the formation of a composition comprising including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof, in said composition.

In a fifth aspect, the present invention still further consists in a hybrid maize seed resulting from a cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

Starch granules from any botanical source are a heterogeneous mixture varying in physiological age and this affects their physical size, structure and properties. If the starch granules are physically separated according to their granule size, it has been noted by a number of authors that the properties of each size fraction are somewhat different. For example, Cluskey et al in Starke, 32, 105–109(1980) reported on the fractionation of dent corn and amylomaize starch granules. They found that an inverse relationship existed between granule size and iodine binding capacity in the amylomaizes. Thus, the percent apparent amylose found in the fractions of amylose V starch amounted to 40% for the largest size particles and 52% for the smallest particles.

The correlation between amylose content and size fraction has been observed by the present inventors in relation to high amylose starches of the type mentioned above and in co-pending patent application PL6537.

In this latter mentioned patent application, PL6537, it was disclosed that high amylose starches have a high dietary fibre or resistant starch content. More specifically, it was found that there was a correlation between amylose content and dietary fibre/resistant starch such that increasing levels of amylose above 55% were associated with increasing levels of dietary fibre/resistant starch.

Patent application PL6537 further disclosed the useful nature of such starches in the preparation of food compositions having an enhanced dietary or resistant starch content.

Based on the observations of

(1) an association of dietary fibre and resistant starch with increasing levels of amylose; and

(2) increasing amylose content with decreasing starch granule size,

it was to be expected that decreasing starch granule size fractions of high amylose starch would be associated with enhanced levels of dietary fibre and resistant starch.

Surprisingly, this was found to be incorrect. In fact it was found that there is an optimum starch granule size fraction which is intermediate in size and not necessarily associated with the highest amylose content fraction.

Accordingly in a sixth aspect, the present invention still further consists in a starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

In a seventh aspect, the present invention still further consists in a food composition having an enhanced dietary fibre and/or resistant starch content, including a starch fraction of enhanced dietary fibre and/or resistant starch content derived from a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

For the purpose of the description of this invention, "high amylose" means an amylose content (dsb) of 50% or more,

preferably 70% or more, most preferably 80% or more. Particularly preferred amylose contents are 85% or more and 90% or more.

For the purposes of the description of the invention, the method by which amylose was determined is set out below.

METHOD: Apparent Amylose (Blue Value)
SCOPE: High Amylose Maize Starch
APPARATUS:
Defatting
Soxhlet extraction apparatus
Steam bath
Whatman thimbles, 25×80 mm
Drying Oven 105° C.
Desiccator
Amylose Determination
Stoppered 50 ml test tubes
Vortex mixer
Boiling water bath
Spectrophotometer (605 mm, slit width 0.2 mm)
REAGENTS:
Defatting
Methanol (AR Grade)
Amylose Determination
Dimethylsulfoxide (HPLC Grade)
Iodine/Potassium iodide solution
3.0 g iodine and 30 g potassium iodide made up to 1000 mls with 0.1N sodium hydroxide
Methanol (AR Grade)
Amylose (Sigma Cat. No AO512)
Dried for 2 hours at 105° C. prior to use.
PROCEDURE:
Defatting
(1) Weigh 5 grams of starch into the thimble.
(2) Place the thimble in the Soxhlet apparatus.
(3) Extract the sample with methanol (200 mls) for 20 hours
(4) Recover the thimble and dry in an oven at 105° C. for 12 hours.
Amylose Determination
(1) Accurately weigh starch (100.0 to 105.0 mg) into the text tube.
(2) Add methanol (1 ml) and vortex mix.
(3) Add DMSO (15 mls), invert the test tube, and vortex mix.
(4) Place the test tubes in a vigorously boiling water bath for 60 minutes.
(5) Invert and vortex mix each test tube at 15 minute intervals during this period.
(6) Add distilled water (15 mls), invert and vortex mix. Place the test tube in the boiling water bath for a further 30 minutes.
(7) Quantitatively transfer the contents of the test tube to a 100 ml volumetric flask (use a funnel in the flask). Make the solution to volume with distilled water.
(8) Transfer an aliquot (3 mls) of this solution to a 100 ml volumetric flask and add 90 mls of distilled water.
(9) Add Iodine/Potassium Iodide solution (1 ml) to the diluted solution and immediately shake and mix thoroughly. Make to volume with distilled water.
(10) Measure the absorbance of this solution at 605 nm compared to a blank consisting of Iodine/Potassium Iodide solution (1 ml) diluted to 100 mls with distilled water in a volumetric flask.

CALCULATIONS:
For native starches:

$$\% \text{ Amylose dsb} = \frac{\text{Absorbance} \times 13}{\text{wt. sample dsb}}$$

* dsb = dry solids basis.

The method by which starch was separated from the maize grain was as follows:

1. Prepare 200 g meal by grinding through the 2 mm then the 1 mm screen of one Retsch Mill.
2. Wet thoroughly, stirring by hand, with 600 ml 0.1N NaOH.
3. Add 2,200 ml 0.1N NaOH and blend 5 minutes at ⅔ speed with the Ultra Turrax.
4. Sieve over 44 u screen.
5. Return sieve overs with 1 L water and blend for another 3 minutes, if necessary.
6. Sieve over 44 u screen.
7. Centrifuge filtrate at 3000 rpm for 15 minutes. Decant. Wipe out the neck of the bottle with a tissue to remove fat.
8. Reslurry starch (centrifugate) with 200 ml water, i.e. 50 ml in each of 4 tubes. Centrifuge.
9. Remove starch from centrifuge tubes with about 250 ml water.
10. Adjust pH of starch slurry to 6.0–6.5 with 0.5N HCl. Filter again over 44 u screen, if necessary.
11. Buchner filter and air dry.

MODES FOR CARRYING OUT THE INVENTION

In order to better understand the nature of this invention, a number of examples will be described.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a gel permeation chromatography molecular weight profile of a number of maize starches;

FIG. 2 is a viscograph of a number of maize starches in water;

FIG. 3 is a viscograph of a number of maize starches in base; and

FIG. 4 is a graph of total dietary fibre versus average starch granule size.

Maize Seed

A range of parental lines of maize seeds were obtained from High Yield Seed Co, Tamworth, Australia. Non-limiting examples of these parenting lines included G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144.

Hybrids were produced by crossing inbred lines carrying the ae amylose extender genes. These inbred lines were selected for combining ability and identified as specific female and male parents to produce the hybrids. Conventional breeding methods and techniques were used in developing inbred lines with repetitive amylose assays to ensure the transfer of recessively inherited ae gene.

One particular cross between male G116 and female G121 resulted in a F1 hybrid, referred to as Code 008 and deposited with the American Type Culture Collection

(ATCC), 12301 Parklawn Drive, Rockville, Md. 20853, U.S.A., under the designation 75182 on Jan. 15, 1992. This hybrid yielded grain the starch of which was found to have an amylose content in excess of 80%.

Based on the disclosure of this invention, the person skilled in the art would expect that hybrids resulting from further crosses of the parental lines mentioned above will yield starch having an amylose content in excess of 80%.

In fact experimental hybrids have yielded starches obtained from crosses between the above mentioned parent lines having high amylose contents. Set out below is a summary of the relevant crosses with amylose content in % bracketed.

	FEMALE	MALE	HYBRID
1.	G117 (81.6)	G116 (82.2)	G117 × G116 (83.3)
2.	G116 (82.2)	G122 (89.6)	G116 × G122 (80.5)
3.	G118 (94.3)	G122 (89.6)	G118 × G122 (85.9)
4.	G120 (94.6)	G122 (89.6)	G120 × G122 (80.4)
5.	G122 (89.6)	G120 (94.6)	G122 × G120 (81.9)
6.	G122 (89.6)	G140 (92.2)	G122 × G140 (85.4)
7.	G128 (71.5)	G129 (61.8)	G128 × G129 (82.8)
8.	G140 (93.2)	G121 (94.7)	G140 × G121 (93.0)
9.	G140 (92.2)	G144 (60.4)	G140 × G144 (85.3)
*10.	G139W (71.9)	G136W (93.4)	G139W × G136W (95.7)
11.	G121 (94.7)	G126 (82.2)	G121 × G116 (85.0)

*W = White seed.

Experiments conducted using Code 008 seed have shown that the climatic and agronomic conditions under which the maize is grown will have a significant effect on the amylose content. Specifically, it has been found that seed cultivated under irrigation near Tamworth, Australia (latitude 31.1° S) in an early crop and a late crop yielded starch having an amylose content respectively of 85.0% and 90.1%. Similarly, a crop cultivated at Finley, Australia (latitude 35.6° S) yielded starch having an amylose content of 94.8%. By contrast, the same seed when cultivated under irrigation at Giru, Australia (latitude 19.5°) yielded a starch having an amylose content of 78.6%.

Accordingly, a preferred embodiment of this invention comprises a maize seed deposited with the ATCC and designated 75182.

A further preferred embodiment of this invention comprises a maize starch having an amylose content of 85.0% or more, most preferably 90.1% or more.

To further characterize the maize starch derived from Code 008 grain, molecular weight profiling by gel permeation chromatography has been performed. The method by which this was done is set out below whilst the results are shown in the accompanying FIG. 1. For comparative purposes, two commercially available maize starches, HA Class V and HA Class VII are shown.

METHOD: Gel Permeation Chromatography of Starch

SCOPE: Starch

APPARATUS:

Sample Preparation

Screw capped test tubes (50 ml)
Boiling water bath
Microcentrifuge (Eppendorf 5415)
Desiccator

HPLC

Column	Alltech GPC High MW Polar 5U (Cat. No. 100586)
Detector	Waters 410 Refractive Index Detector (× 128 35° C.)
Pump	Waters 600 E
Injector	Waters 712 WISP
Column Heater	(Set at 25° C.)
Software	Maxima 825 (V 3.3)

REAGENTS:

Dimethyl sulfoxide (Chrom AR HPLC Grade - Mallinckrodt)
Dimethyl formamide (Chrom AR HPLC Grade - Mallinckrodt)
Pullulan Molecular Weight Standards - Shown
Denko (ex Edward Instruments)
HPLC Mobile Phase - DMSO:DMF (20:80)
SAMPLE PREPARATION:

Standards

(1) The pullulan molecular weight standards need to be weighed into the screw capped test tubes in the following manner:
Tube 1 - 5.0 mg each of P800, P100, P10 and glucose
Tube 2 - 7.0 mg each of P400, P50 and P5
Tube 3 - 7.0 mg each of P200, P20 and maltotriose.
(2) Add DMSO (4 mls) to each tube and tightly seal it.
(3) heat the tubes in the boiling water bath for 5 minutes to dissolve the pullulan.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (16 mls) and mix well.
(6) Place 3 × 1.5 ml aliquots into microcentrifuge tubes and centrifuge at 14000 rpm for 10 minutes.
(7) Remove the top 1 ml of solution from each centrifuge tube and place in a WISP vial.

Samples

(1) Accurately weight the sample (50.0 mg) into a screw capped test tube.
(2) Add DMSO (10 mls).
(3) Heat in a boiling water bath for 60 minutes.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (40 mls) and mix well.
(6) Place 3 × 1.5 ml aliquots into microcentrifuge tubes and centrifuge at 14000 rpm for 10 minutes.
(7) Remove the top 1 ml of solution from each centrifuge tube and place in a WISP vial.

HPLC Preparation

(1) Prior to fitting the column, pump water (100 mls) through the HPLC.
(2) Prepare the mobile phase and pump 50 mls through the HPLC. Ensure that the WISP is purged during this stage.
(3) Adjust the flow rate of 0.2 ml/minute and connect the column.
(4) Allow the column to equilibrate overnight.
(5) Prior to the injection of samples, purge the WISP and then gradually increase the flow rate to 1.5 mls/minute.
(6) Set the column heater to 25° C.
(7) Inject the standards and samples - 100 µl injection volume.
(8) After samples have been analysed turn the column heater off and reduce the flow rate of 0.2 mls/minute

-continued

(9)	Disconnect the column.
(10)	Wash the system with water overnight at 0.5 mls/minute.
(11)	Wash the system with methanol (200 mls).

Viscographs have also been prepared comparing maize starch from Code 008 (designated Gelose 80) with Gelose 50 and Gelose 70. FIG. 2 shows the viscosity profile under alkaline conditions whilst FIG. 3 shows the viscosity profile in water.

Maize Starch

The maize starch of the first aspect of this invention having an amylose content of more than 80% may be used in a variety of compositions known in the art. The usefulness of the starch is believed to be a result of the higher content of more linear molecules. This seems to impart physical properties which tend towards those of conventionally used synthetic plastics materials. Consequently, films formed from the starch of the invention have higher tensile strengths and are good oxygen barriers. The starch is also easier to process on existing synthetic plastics materials equipment such as blow moulding and injection moulding machines.

Furthermore, this starch may be physically modified or chemically modified to produce a variety of derivatives well known in the art. These starches may also be used in a variety of compositions.

Finally, this starch may also be used in processes and compositions requiring the starch to be destructurized within the meaning of that term defined in EP0118240.

Some non-limiting examples of compositions in which the maize starch of this invention in all of its forms, could be used include:

1. Corrugating adhesives.
2. Sausage skins.
3. Confectionery.
4. Other food compositions where the enhanced gel strength of the starch would be advantageous.
5. Films, either alone or laminated with polymers such as ethylenevinylalcohol to achieve both gas and water barrier properties.
6. Biodegradable and controlled release matrices and methods for forming and using these matrices as disclosed in PCT/AU90/00422, the contents of which is incorporated herein by way of reference.
7. Shaped articles, processes for forming shaped articles and methods for using shaped articles as disclosed in PCT/AU90/00237, the contents of which is incorporated herein by way of reference.
8. Coextrusions with synthetic polymers.
9. Intermediate products such as pellets and rods, formed for example by extrusion, and including combinations of starch with one or more natural or synthetic polymers, plasticizers, colourants and other additives.
10. Other blends of starch with natural or synthetic polymers to obtain enhanced structural properties.

Starch Fractions

The starches of the sixth and seventh aspects of this invention may originate from a number of sources including cereals such as maize, barley, wheat and legumes, providing that the starch content of such sources is high in amylose.

To fractionate the starch granules, there are a number of methods known in the art including dry powder sieving, hydrocyclone classification, air classification and differential sedimentation. A person skilled in the art would be readily able to choose an appropriate method depending on the source material and other relevant factors.

Although the size fraction of enhanced dietary fibre and/or resistant starch may vary, the example that follows describes the work that was done by the present inventors in relation to a maize starch sample. Based on this disclosure, a person skilled in the art could readily repeat this work using other starch sources to identify an appropriate fraction.

Once the starch has been appropriately fractionated, the fractions having enhanced dietary fibre and/or resistant starch content may be processed to obtain starch having further increased dietary fibre and/or resistant starch content using entirely conventional methods well known in the art. An example of the fractionation will now be described.

Fractionation of Maize Starch by Granule Size

A high amylose maize starch—High Amylose 80(10/91) was fractionated into seven subsamples based on granule size using the aqueous differential sedimentation procedure described by Cluskey et al (1980). This method was chosen since it minimised damage to the starch, did not introduce any residues and it was indicated that exposure of the starch granules to distilled water for long periods of time did not affect their integrity. Each subsample was weighed, measured for average granule size and the apparent amylose content, total dietary fibre and resistant starch determined. Each starch sample (60 grams) was separated into the seven fractions which were freeze-dried and weighed on a Mettler PE 3600 top pan balance. A scanning electron microscope was used to visually check the uniformity of the size distribution of the granules in each fraction.

Each fractionated starch sample was analysed for granule size according to the method described below. Apparent amylose content was determined using the method described above. Dietary fibre and resistant starch (McCleary et al) were determined using the methods disclosed in co-pending application PL6537.

Granule size was determined using a Malvern Master Sizer which utilises a He-Ne laser (632.8 nm) with a maximum output of 5mW CW. In this method a starch slurry was made using approximately 15 mL of distilled water in a 50 mL beaker. The slurry was sonicated for 4 minutes. The slurry was then introduced into the stirred cell and the obscuration value adjusted using distilled water to 0.20. The slurry was allowed to stir for a further 2 minutes before readings were taken. Four readings were taken for each sample in order to check the stability of the readings being obtained.

Results

In Table 1 set out below, there is shown the results (the average of two separate fractionations, together with the range of analytical results) obtained for each of seven particle size fractions. These results are graphically presented in FIG. 4, from which it is particularly evident that the level of resistant starch and dietary fibre is significantly increased between the second and fifth fractions, ie, 10.2–7.6 microns. Thus, if those starch fractions were to be segregated from the original starch sample, only 46.9% of the solids would need to be removed to produce a fraction in which the resistant starch was increased by 36% and dietary fibre by 24%.

Although the starch fractions of the invention are high in dietary fibre and/or resistant starch, it should also be appreciated that another important property is that these fractions are "naturally" derived. This arises out of the fact that the fractions are prepared using a physical means of separation. No chemical or other treatments are required in order to produce starch fractions having a high dietary fibre and/or resistant starch content. Such a property is of particular importance in food applications in that no regulatory approval would be required in order to incorporate such materials in food compositions.

TAELE 1

Fractionation of High Amylose 80 (10/91) Maize Starch by Granule Size

	Amount in Fraction (%) dsb	Average Granule Size (microns)	Apparent Amylose Content (%) dsb	Total Dietary Fibre (%) dsb	Resistant Starch (%) dsb
High Amylose 80 - 10/91	100.00	10.0	85	33.4	18.1
Fraction 1	35.6 ± 1.1	12.3 ± 0.5	80 ± 0	31.4 ± 1.5	17.7
Fraction 2	15.0 ± 2.6	10.2 ± 0.1	83 ± 1	38.3 ± 2.0	16.4
Fraction 3	13.0 ± 1.1	9.1 ± 0.2	85.5 ± 0.5	41.3 ± 0.3	22.8
Fraction 4	14.9 ± 1.0	8.3 ± 0.1	85.5 ± 0.5	39.4 ± 4.1	24.6
Fraction 5	10.2 ± 1.6	7.6 ± 0.1	88.5 ± 0.5	37.2 ± 1.3	18.9
Fraction 6	7.0 ± 1.6	7.2 ± 0.1	89.5 ± 0.5	31.3 ± 2.4	21.7
Fraction 7	4.3 ± 2.7	6.8 ± 0.2	89	28.1	10.1

The person skilled in the art will readily appreciate that the starch fractions of the invention having the enhanced dietary fibre and/or resistant starch content may be used in a variety of food compositions. Such uses are disclosed, for example, in co-pending application No PL6537.

Whilst it is not as yet known why the fractions of the invention have enhanced dietary fibre and/or resistant starch content, it will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described without departing from the spirit or scope of the invention as broadly described. Accordingly, the Example based on a sample of high amylose maize starch is to be considered in all respects as illustrative and not restrictive.

The person skilled in the art will readily appreciate that the maize starch of the invention both in its native form, and the other forms mentioned above will have many applications additional to those mentioned.

It will also be appreciated by those skilled in the art that numerous variations and modifications may be made to this invention without departing from the spirit or scope thereof as broadly described.

What is claimed is:

1. A maize starch selected from the group consisting of maize starch having an apparent amylose content of more than 90.1%, physically or chemically modified derivatives of maize starch having an apparent amylose content of more than 90.1%, destructurized maize starch having an apparent amylose content of more than 90.1%, and non-destructurized maize starch having an apparent amylose content of more than 90.1%.

2. A maize starch as in claim 1 having an apparent amylose content of 95.7% or more.

3. A maize starch as in claim 1 having an apparent amylose content of 93.0%.

4. A composition comprising a maize starch selected from the group consisting of maize starch having an apparent amylose content of more than 90.1%, physically or chemically modified derivatives of maize starch having an apparent amylose content of more than 90.1%, destructurized maize starch having an apparent amylose content of more than 80%, and non-destructurized maize starch having an apparent amylose content of more than 90.1%.

5. A composition as in claim 4 wherein the maize starch has an apparent amylose content of 95.7% or more.

* * * * *

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PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

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PTO-1556
(5/87)

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Technical Field

This invention relates to high amylose content starch, in particular to a maize starch having an amylose content of more than 80% w/w. The invention further relates to single, double and multiple cross maize hybrids, particularly to a maize single cross F1 hybrid, capable of producing grain having such a high amylose content and to this grain.

The invention still further relates to fractions of high amylose starch that are enriched in dietary fibre and resistant starch content whilst claiming a high amylose content.

Background Art

Most common starches contain approximately 25% amylose and 75% amylopectin. Amylose is a linear glucose polymer fraction, whilst amylopectin is a branched glucose polymer fraction.

In the prior art, it has been recognized that currently available commercial starch having an elevated amylose content would impart certain desirable properties to various compositions including films, foods and industrial products. Accordingly, attempts have been made in the prior art to produce high amylose content maize. This is exemplified in AU-A-45616/89 wherein a maize seed deposited as ATCC No. 40499 is disclosed as capable of yielding a starch having an amylose content of up to 72%.

Typically, however, a commercial starch having an amylose content of 55-65% would be regarded in the art as having a high amylose content.

The present inventors whilst recognizing the utility of the commercially available so-called high amylose starches, have sought to produce a maize having a still higher amylose content.

Disclosure of Invention

In the course of a breeding program, a single cross F1 hybrid maize seed was produced, which carried the ae amylose extender gene. This seed was found to be capable of producing grain, in which the amylose content of the starch derived therefrom was in excess of 80%.

Accordingly, in a first aspect, this invention consists in a hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

In a second aspect, this invention further consists in a maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

In a third aspect, this invention still further consists in compositions including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

In a fourth aspect, this invention still further consists in a process for the formation of a composition comprising including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof, in said composition.

In a fifth aspect, the present invention still further consists in a hybrid maize seed resulting from a cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

Starch granules from any botanical source are a heterogeneous mixture varying in physiological age and this affects their physical size, structure and properties. If the starch granules are physically separated according to their granule size, it has been noted by a number of authors that the properties of each size fraction are somewhat different. For example, Cluskey et al in Starke, 32, 105-109(1980) reported on the fractionation of dent corn and amylomaize starch granules. They found that an inverse relationship existed between granule size and iodine binding capacity in the amylomaizes. Thus, the percent apparent amylose found in the fractions of amylose V starch amounted to 40% for the largest size particles and 52% for the smallest particles.

The correlation between amylose content and size fraction has been observed by the present inventors in relation to high amylose starches of the type mentioned above and in co-pending patent application PL6537.

In this latter mentioned patent application, PL6537, it was disclosed that high amylose starches have a high dietary fibre or resistant starch content. More specifically, it was found that there was a correlation between amylose content and dietary fibre/resistant starch such that increasing levels of amylose above 55% were associated with increasing levels of dietary fibre/resistant starch.

Patent application PL6537 further disclosed the useful nature of such starches in the preparation of food compositions having an enhanced dietary or resistant starch content.

Based on the observations of

(1) an association of dietary fibre and resistant starch with increasing levels of amylose; and

(2) increasing amylose content with decreasing starch granule size,

it was to be expected that decreasing starch granule size fractions of high amylose starch would be associated with enhanced levels of dietary fibre and resistant starch.

Surprisingly, this was found to be incorrect. In fact it was found that there is an optimum starch granule size fraction which is intermediate in size and not necessarily associated with the highest amylose content fraction.

Accordingly in a sixth aspect, the present invention still further consists in a starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

In a seventh aspect, the present invention still further consists in a food composition having an enhanced dietary fibre and/or resistant starch content, including a starch fraction of enhanced dietary fibre and/or resistant starch content derived from a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

For the purpose of the description of this invention, "high amylose" means an amylose content (dsb) of 50% or more, preferably 70% or more, most preferably 80% or more. Particularly preferred amylose contents are 85% or more and 90% or more.

For the purposes of the description of the invention, the method by which amylose was determined is set out below.

METHOD: Apparent Amylose (Blue Value)

SCOPE: High Amylose Maize Starch

APPARATUS:

Defatting

- Soxhlet extraction apparatus
- Steam bath
- Whatman thimbles, 25 x 80mm
- Drying Oven 105°C
- Desiccator

Amylose Determination

- Stoppered 50ml test tubes
- Vortex mixer
- Boiling water bath
- Spectrophotometer (605mm, slit width 0.2mm)

REAGENTS:

Defatting

- Methanol (AR Grade)

Amylose Determination

- Dimethylsulfoxide (HPLC Grade)
- Iodine/Potassium iodide solution
  - 3.0g iodine and 30g potassium iodide made up to 1000mls with 0.1N sodium hydroxide
- Methanol (AR Grade)
- Amylose (Sigma Cat. No A0512)
  - Dried for 2 hours at 105°C prior to use.

PROCEDURE:

Defatting

(1) Weigh 5 grams of starch into the thimble.

(2) Place the thimble in the Soxhlet apparatus.

(3) Extract the sample with methanol (200mls) for 20 hours

(4) Recover the thimble and dry in an oven at 105°C for 12 hours.

Amylose Determination

(1) Accurately weigh starch (100.0 to 105.0mg) into the text tube.

(2) Add methanol (1ml) and vortex mix.

(3) Add DMSO (15mls), invert the test tube, and vortex mix.

(4) Place the test tubes in a vigorously boiling water bath for 60 minutes.

(5) Invert and vortex mix each test tube at 15 minute intervals during this period.

(6) Add distilled water (15mls), invert and vortex mix. Place the test tube in the boiling water bath for a further 30 minutes.

(7) Quantitatively transfer the contents of the test tube to a 100ml volumetric flask (use a funnel in the flask). Make the solution to volume with distilled water.

(8) Transfer an aliquot (3mls) of this solution to a 100ml volumetric flask and add 90mls of distilled water.

(9) Add Iodine/Potassium Iodide solution (1ml) to the diluted solution and immediately shake and mix thoroughly. Make to volume with distilled water.

(10) Measure the absorbance of this solution at 605 nm compared to a blank consisting of Iodine/ Potassium Iodide solution (1ml) diluted to 100mls with distilled water in a volumetric flask.

CALCULATIONS:

For native starches:

$$\% \text{ Amylose dsb} = \frac{\text{Absorbance} \times 13}{\text{wt. sample dsb}}$$

* dsb = dry solids basis.

The method by which starch was separated from the maize grain was as follows:-

1. Prepare 200g meal by grinding through the 2mm then the 1mm screen of the Retsch Mill.
2. Wet thoroughly, stirring by hand, with 600ml 0.1N NaOH.
3. Add 2,200ml 0.1N NaOH and blend 5 minutes at 2/3 speed with the Ultra Turrax.
4. Sieve over 44u screen.
5. Return sieve overs with 1L water and blend for another 3 minutes, if necessary.
6. Sieve over 44u screen.
7. Centrifuge filtrate at 3000 rpm for 15 minutes. Decant. Wipe out the neck of the bottle with a tissue to remove fat.
8. Reslurry starch (centrifugate) with 200ml water, i.e. 50ml in each of 4 tubes. Centrifuge.
9. Remove starch from centrifuge tubes with about 250ml water.
10. Adjust pH of starch slurry to 6.0-6.5 with 0.5N HCl. Filter again over 44u screen, if necessary.
11. Buchner filter and air dry.

Modes for Carrying out the Invention

In order to better understand the nature of this invention, a number of examples will be described.

Brief Description of Drawings

Fig. 1 is a gel permeation chromatography molecular weight profile of a number of maize starches;

Fig. 2 is a viscograph of a number of maize starches in water;

Fig. 3 is a viscograph of a number of maize starches in base; and

Fig. 4 is a graph of total dietary fibre versus average starch granule size.

Maize Seed

A range of parental lines of maize seeds were obtained from High Yield Seed Co, Tamworth, Australia. Non-limiting examples of these parenting lines included

G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144.

Hybrids were produced by crossing inbred lines carrying the <u>ae</u> amylose extender genes. These inbred lines were selected for combining ability and identified as specific female and male parents to produce the hybrids. Conventional breeding methods and techniques were used in developing inbred lines with repetitive amylose assays to ensure the transfer of recessively inherited <u>ae</u> gene.

One particular cross between male G116 and female G121 resulted in a F1 hybrid, referred to as Code 008 and deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD20853, U.S.A., under the designation 75182 on 15 January 1992. This hybrid yielded grain the starch of which was found to have an amylose content in excess of 80%.

Based on the disclosure of this invention, the person skilled in the art would expect that hybrids resulting from further crosses of the parental lines mentioned above will yield starch having an amylose content in excess of 80%.

In fact experimental hybrids have yielded starches obtained from crosses between the above mentioned parent lines having high amylose contents. Set out below is a summary of the relevant crosses with amylose content in % bracketed.

	FEMALE	MALE	HYBRID
1.	G117 (81.6)	G116 (82.2)	G117 x G116 (83.3)
2.	G116 (82.2)	G122 (89.6)	G116 x G122 (80.5)
3.	G118 (94.3)	G122 (89.6)	G118 x G122 (85.9)
4.	G120 (94.6)	G122 (89.6)	G120 x G122 (80.4)
5.	G122 (89.6)	G120 (94.6)	G122 x G120 (81.9)
6.	G122 (89.6)	G140 (92.2)	G122 x G140 (85.4)
7.	G128 (71.5)	G129 (61.8)	G128 x G129 (82.8)
8.	G140 (93.2)	G121 (94.7)	G140 x G121 (93.0)
9.	G140 (92.2)	G144 (60.4)	G140 x G144 (85.3)
* 10.	G139W (71.9)	G136W (93.4)	G139W x G136W (95.7)
11.	G121 (94.7)	G126 (82.2)	G121 x G116 (85.0)

* W = White seed.

Experiments conducted using Code 008 seed have shown that the climatic and agronomic conditions under which the maize is grown will have a significant effect on the amylose content. Specifically, it has been found that seed cultivated under irrigation near Tamworth, Australia (latitude 31.1°S) in an early crop and a late crop yielded starch having an amylose content respectively of 85.0% and 90.1%. Similarly, a crop cultivated at Finley, Australia (latitude 35.6°S) yielded starch having an amylose content of 94.8%. By contrast, the same seed when cultivated under irrigation at Giru, Australia (latitude 19.5°) yielded a starch having an amylose content of 78.6%.

Accordingly, a preferred embodiment of this invention comprises a maize seed deposited with the ATCC and designated 75182.

A further preferred embodiment of this invention comprises a maize starch having an amylose content of 85.0% or more, most preferably 90.1% or more.

To further characterize the maize starch derived from Code 008 grain, molecular weight profiling by gel

permeation chromatography has been performed. The method by which this was done is set out below whilst the results are shown in the accompanying Figure 1. For comparative purposes, two commercially available maize starches, HA Class V and HA Class VII are shown.

METHOD: Gel Permeation Chromatography of Starch

SCOPE: Starch

APPARATUS:

Sample Preparation

Screw capped test tubes (50ml)
Boiling water bath
Microcentrifuge (Eppendorf 5415)
Desiccator

HPLC

Column	Alltech GPC High MW Polar 5U (Cat. No. 100586)
Detector	Waters 410 Refractive Index Detector (X 128 35°C)
Pump	Waters 600 E
Injector	Waters 712 WISP
Column Heater	(Set at 25°C)
Software	Maxima 825 (V 3.3)

REAGENTS:

Dimethyl sulfoxide (Chrom AR HPLC Grade - Mallinckrodt)
Dimethyl formamide (Chrom AR HPLC Grade - Mallinckrodt)
Pullulan Molecular Weight Standards - Showa Denko (ex Edward Instruments)

HPLC Mobile Phase - DMSO:DMF (20:80)

SAMPLE PREPARATION:

Standards

(1) The pullulan molecular weight standards need to be weighed into the screw capped test tubes in the following manner:

Tube 1 - 5.0mg each of P800, P100, P10 and glucose
Tube 2 - 7.0mg each of P400, P50 and P5
Tube 3 - 7.0mg each of P200, P20 and maltotriose.

(2) Add DMSO (4mls) to each tube and tightly seal it.
(3) heat the tubes in the boiling water bath for 5 minutes to dissolve the pullulan.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (16mls) and mix well.
(6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
(7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

Samples

(1) Accurately weight the sample (50.0mg) into a screw capped test tube.
(2) Add DMSO (10mls).
(3) Heat in a boiling water bath for 60 minutes.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (40mls) and mix well.
(6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
(7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

HPLC Preparation

(1) Prior to fitting the column, pump water (100mls) through the HPLC.
(2) Prepare the mobile phase and pump 50mls through the HPLC. Ensure that the WISP is purged during this stage.
(3) Adjust the flow rate of 0.2ml/minute and connect the column.
(4) Allow the column to equilibrate overnight.

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(5) Prior to the injection of samples, purge the WISP and then gradually increase the flow rate to 1.5mls/minute.

(6) Set the column heater to 25°C.

(7) Inject the standards and samples - 100µl injection volume.

(8) After samples have been analysed turn the column heater off and reduce the flow rate of 0.2mls/minute.

(9) Disconnect the column.

(10) Wash the system with water overnight at 0.5mls/minute.

(11) Wash the system with methanol (200mls).

Viscographs have also been prepared comparing maize starch from Code 008 (designated Gelose 80) with Gelose 50 and Gelose 70. Figure 2 shows the viscosity profile under alkaline conditions whilst Figure 3 shows the viscosity profile in water.

Maize Starch

The maize starch of the first aspect of this invention having an amylose content of more than 80% may be used in a variety of compositions known in the art. The usefulness of the starch is believed to be a result of the higher content of more linear molecules. This seems to impart physical properties which tend towards those of conventionally used synthetic plastics materials. Consequently, films formed from the starch of the invention have higher tensile strengths and are good oxygen barriers. The starch is also easier to process on existing synthetic plastics materials equipment such as blow moulding and injection moulding machines.

Furthermore, this starch may be physically modified or chemically modified to produce a variety of derivatives well known in the art. These starches may also be used in a variety of compositions.

Finally, this starch may also be used in processes and compositions requiring the starch to be destructurized within the meaning of that term defined in EP0118240.

Some non-limiting examples of compositions in which the maize starch of this invention in all of its forms, could be used include:

1. Corrugating adhesives.
2. Sausage skins.
3. Confectionery.
4. Other food compositions where the enhanced gel strength of the starch would be advantageous.
5. Films, either alone or laminated with polymers such as ethylenevinylalcohol to achieve both gas and water barrier properties.
6. Biodegradable and controlled release matrices and methods for forming and using these matrices as disclosed in PCT/AU90/00422, the contents of which is incorporated herein by way of reference.
7. Shaped articles, processes for forming shaped articles and methods for using shaped articles as disclosed in PCT/AU90/00237, the contents of which is incorporated herein by way of reference.
8. Coextrusions with synthetic polymers.
9. Intermediate products such as pellets and rods, formed for example by extrusion, and including combinations of starch with one or more natural or synthetic polymers, plasticizers, colourants and other additives.
10. Other blends of starch with natural or synthetic polymers to obtain enhanced structural properties.

Starch Fractions

The starches of the sixth and seventh aspects of this invention may originate from a number of sources including cereals such as maize, barley, wheat and legumes, providing that the starch content of such sources is high in amylose.

To fractionate the starch granules, there are a number of methods known in the art including dry powder sieving, hydrocyclone classification, air classification and differential sedimentation. A person skilled in the art would be readily able to choose an appropriate method depending on the source material and other relevant factors.

Although the size fraction of enhanced dietary fibre and/or resistant starch may vary, the example that follows describes the work that was done by the present inventors in relation to a maize starch sample. Based on this disclosure, a person skilled in the art could readily repeat this work using other starch sources to identify an appropriate fraction.

Once the starch has been appropriately fractionated, the fractions having enhanced dietary fibre and/or resistant starch content may be processed to obtain starch having further increased dietary fibre and/or resistant starch content using entirely conventional methods well known in the art. An example of the fractionation will now be described.

Fractionation of Maize Starch by Granule Size

A high amylose maize starch - High Amylose 80(10/91) was fractionated into seven subsamples based on granule size using the aqueous differential sedimentation procedure described by Cluskey et al (1980). This method was chosen since it minimised damage to the starch, did not introduce any residues and it was indicated that exposure of the starch granules to distilled water for long periods of time did not affect their integrity. Each subsample was weighed, measured for average granule size and the apparent amylose content, total dietary fibre and resistant starch determined. Each starch sample (60 grams) was separated into the seven fractions which were freeze-dried and weighed on a Mettler PE 3600 top pan

balance. A scanning electron microscope was used to visually check the uniformity of the size distribution of the granules in each fraction.

Each fractionated starch sample was analysed for granule size according to the method described below. Apparent amylose content was determined using the method described above. Dietary fibre and resistant starch (McCleary et al) were determined using the methods disclosed in co-pending application PL6537.

Granule size was determined using a Malvern Master Sizer which utilises a He-Ne laser (632.8nm) with a maximum output of 5mW CW. In this method a starch slurry was made using approximately 15mL of distilled water in a 50mL beaker. The slurry was sonicated for 4 minutes. The slurry was then introduced into the stirred cell and the obscuration value adjusted using distilled water to 0.20. The slurry was allowed to stir for a further 2 minutes before readings were taken. Four readings were taken for each sample in order to check the stability of the readings being obtained.

Results

In Table 1 set out below, there is shown the results (the average of two separate fractionations, together with the range of analytical results) obtained for each of seven particle size fractions. These results are graphically presented in Fig.4, from which it is particularly evident that the level of resistant starch and dietary fibre is significantly increased between the second and fifth fractions, ie, 10.2-7.6 microns. Thus, if those starch fractions were to be segregated from the original starch sample, only 46.9% of the solids would need to be removed to produce a fraction in which the resistant starch was increased by 36% and dietary fibre by 24%.

Although the starch fractions of the invention are

TABLE 1

Fractionation of High Amylose 80 (10/91) Maize Starch by Granule Size

	Amount in Fraction (I) dsb	Average Granule Size (microns)	Apparent Amylose Content (I) dsb	Total Dietary Fibre (I) dsb	Resistant Starch (I) dsb
High Amylose 80 - 10/91	100.00	10.0	85	33.4	18.1
Fraction 1	35.6 ± 1.1	12.3 ± 0.5	80 ± 0	31.4 ± 1.5	17.7
Fraction 2	15.0 ± 2.6	10.2 ± 0.1	83 ± 1	38.3 ± 2.0	16.4
Fraction 3	13.0 ± 1.1	9.1 ± 0.2	85.5 ± 0.5	41.3 ± 0.3	22.8
Fraction 4	14.9 ± 1.0	8.3 ± 0.1	85.5 ± 0.5	39.4 ± 4.1	24.6
Fraction 5	10.2 ± 1.6	7.6 ± 0.1	88.5 ± 0.5	37.2 ± 1.3	18.9
Fraction 6	7.0 ± 1.6	7.2 ± 0.1	89.5 ± 0.5	31.3 ± 2.4	21.7
Fraction 7	4.3 ± 2.7	6.8 ± 0.2	89	28.1	10.1

high in dietary fibre and/or resistant starch, it should also be appreciated that another important property is that these fractions are "naturally" derived. This arises out of the fact that the fractions are prepared using a physical means of separation. No chemical or other treatments are required in order to produce starch fractions having a high dietary fibre and/or resistant starch content. Such a property is of particular importance in food applications in that no regulatory approval would be required in order to incorporate such materials in food compositions.

The person skilled in the art will readily appreciate that the starch fractions of the invention having the enhanced dietary fibre and/or resistant starch content may be used in a variety of food compositions. Such uses are disclosed, for example, in co-pending application No PL6537.

Whilst it is not as yet known why the fractions of the invention have enhanced dietary fibre and/or resistant starch content, it will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described without departing from the spirit or scope of the invention as broadly described. Accordingly, the Example based on a sample of high amylose maize starch is to be considered in all respects as illustrative and not restrictive.

The person skilled in the art will readily appreciate that the maize starch of the invention both in its native form, and the other forms mentioned above will have many applications additional to those mentioned.

It will also be appreciated by those skilled in the art that numerous variations and modifications may be made to this invention without departing from the spirit or scope thereof as broadly described.

CLAIMS:

1. A hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

2. A hybrid maize seed as in claim 1 obtained from a cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

3. A hybrid maize seed as in claim 2 selected from the group consisting of the following crosses: G117 x G116, G116 x G122, G118 x G122, G120 x G122, G112 x G120, G122 x G140, G128 x G129, G140 x G121, G140 x G144, G139W x G136W and G121 x G116.

4. A hybrid maize seed as in any one of claims 1 to 3 wherein the seed yields a starch having an amylose content of 85.0% or more.

5. A hybrid maize seed as in claim 4 wherein the seed yields a starch having an amylose content of 90.1% or more.

6. A hybrid maize seed as in claim 5 wherein the seed yields a starch having an amylose content of 94.8% or more.

7. A hybrid maize seed as in claim 3 deposited as ATCC 75182.

8. A maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

9. A maize starch as in claim 8 having an amylose content of 85.0% or more.

10. A maize starch as in claim 9 having an amylose content of 90.1% or more.

11. A maize starch as in claim 10 having an amylose content of 94.8% or more.

12. A composition including a maize starch selected from

the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

13. A composition as in claim 12 wherein the maize starch has an amylose content of 85.0% or more.

14. A composition as in claim 13 wherein the maize starch has an amylose content of 90.1% or more.

15. A composition as in claim 14 wherein the maize starch has an amylose content of 94.8% or more.

16. A starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch, the amylose content of which is 50% or more, which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

17. A starch fraction as in claim 16 wherein the high amylose starch is selected from the group consisting of maize, barley, wheat and legumes.

18. A starch fraction as in claim 16 or claim 17 wherein the amylose content of the high amylose starch is 70% or more, preferably 80% or more.

19. A starch fraction as in claim 18 wherein the amylose content of the high amylose starch is 85% or more, preferably 90% or more.

20. A starch fraction as in any one of claims 16 to 19 wherein the fractionation is by dry powder sieving, hydrocyclone classification, air classification or differential sedimentation.

21. A starch fraction as in any one of claims 16 to 20 wherein the dietary fibre content of the fraction is increased by about 24% or more and the resistant starch content of the fraction is increased by about 36% or more over the high amylose starches.

22. A starch fraction as in any one of claims 16 to 21 wherein the average granule size of the fraction is from about 10.2 to 7.6 microns.

23. A food composition including a starch fraction as claimed in any one of claims 16 to 22.

Add 61
Add 62
Add 63

Add 64

DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled , the specification of which HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

[ ] is attached hereto [X] was filed on as Application Serial No. and was amended on (if applicable)

PCT/AU93/00389 filed 30 July 1993

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I hereby appoint the following attorney(s) and/or agent(s): Allan M. Lowe, Reg. No. 19,641; Robert L. Price, Reg. No. 22,685; Robert E. LeBlanc, Reg. No. 17,219; Stephen A. Becker, Reg. No. 26,527; Henry Shur, Reg. No. 17,414; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Donald C. Casey, Reg. No. 24,022; Kenneth E. Krosin, Reg. No. 25,735; Chittaranjan N. Nirmel, Reg. No. 30,408; Holly D. Kozlowski, Reg. No. 30,468; Gene Z. Rubinson, Reg. No. 33,351; Frank P. Presta; Reg. No. 19,828; Michael S. Gzybowski, Reg. No. 32,816; Robert G. Lev, Reg. No. 30,280; Keith E. George, Reg. No. 34,111; Arthur P. Demers, Reg. No. 32,660; Edward J. Wise, Reg. No. 34,523; Christopher W. Brody, Reg. No. 33,613; Demetra J. Mills, Reg. No. 34,506; Daniel Y.J. Kim, Reg. No. 36,186; Alexander Yampolsky, Reg. No. 36,324; Sharon E. Finkel, Reg. No. 35,798; Robert P. Bell, Reg. No. 34,546; and Alfred A. Stadnicki, Reg. No. 30,226: all of

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with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

Full name of sole or first inventor: Kenneth J McNAUGHT
Inventor's Signature: Kenneth McNaught Date: 13 January 1995
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PCT/AU93/00389 filed 30 July 1993

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Full Name of Second Inventor: Eric MALONEY

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Third Inventor: Ian L BROWN

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

65010

DECLARATION, POWER OF ATTORNEY AND PETITION

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My residence, post office and citizenship are as stated below next to my name,

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Full name of sole or first inventor: Kenneth J McNAUGHT

Inventor's Signature _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

65010

page 2 of 2

PCT/AU93/00389 filed 30 July 1993

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Full Name of Second Inventor: Eric ~~MAHONEY~~ MOLONEY E.R.M. 11/4/94

Inventor's Signature: E.R.Moloney Date: 22/12/94.

Residence: 169 Brisbane Street, Tamworth, NSW 2340, Australia

Citizenship: Australian

Post Office Address: As above

Full Name of Third Inventor: Ian L BROWN

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

05 070

DECLARATION, POWER OF ATTORNEY AND PETITION

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Full name of sole or first inventor: Kenneth J McNAUGHT

Inventor's Signature _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

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Full Name of Second Inventor: Eric MALONEY

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Third Inventor: Ian L BROWN

Inventor's Signature: _____ Date: 15/12/94

Residence: 2 Melissa Avenue, Tamworth, NSW 2340, Australia

Citizenship: Australian

Post Office Address: As above

Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

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Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Third Inventor: Ian L BROWN

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: A T Knight Date: 30/11/94

Residence: 18 Nundah Street, Lane Cove, NSW 2066, Australia

Citizenship: Australian

Post Office Address: As above

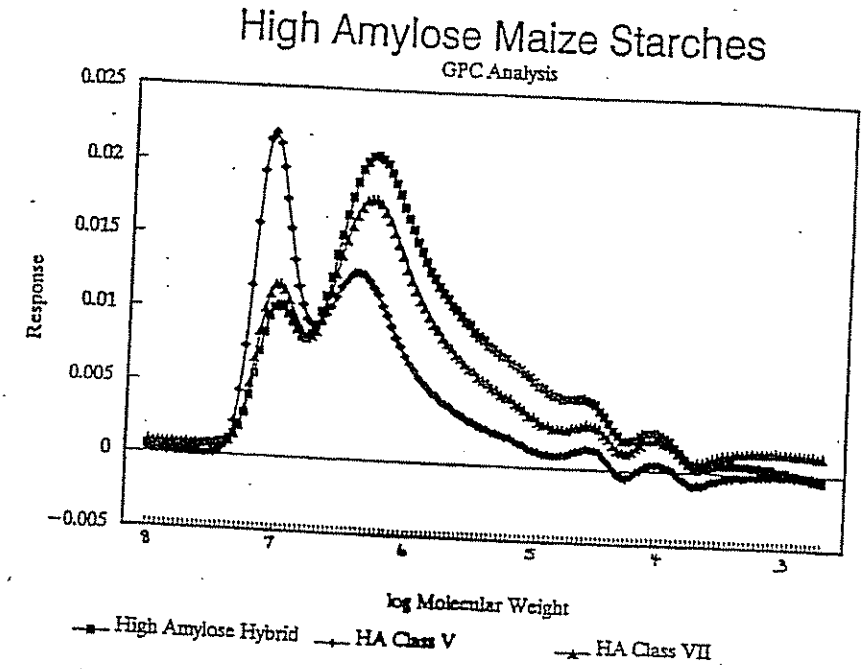


FIG. 1

2/4

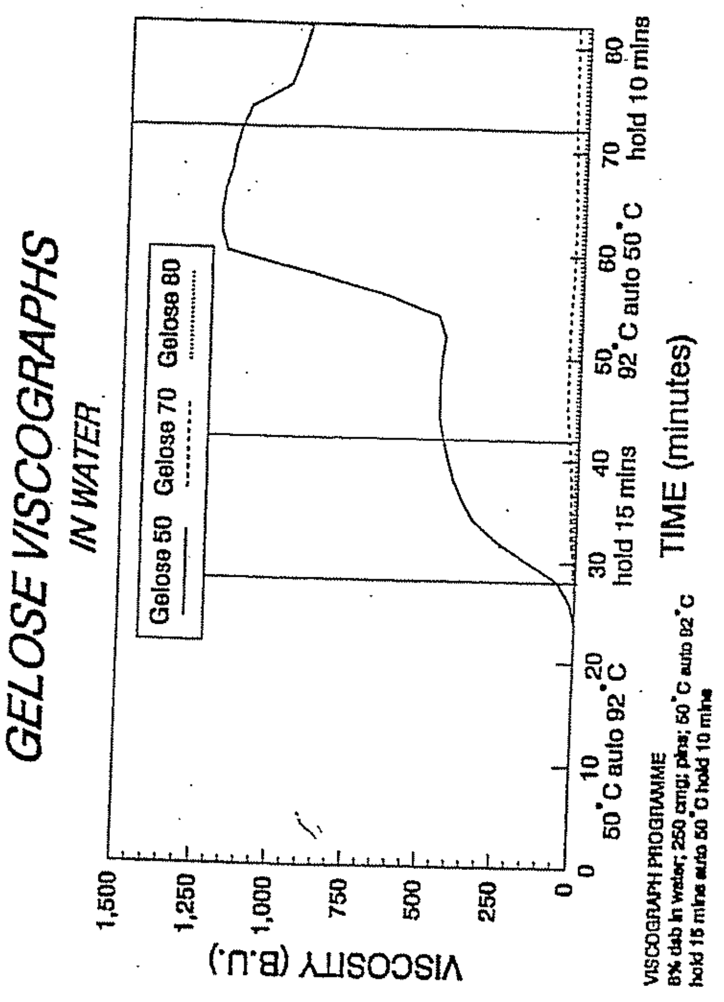


FIG. 2

SUBSTITUTE SHEET

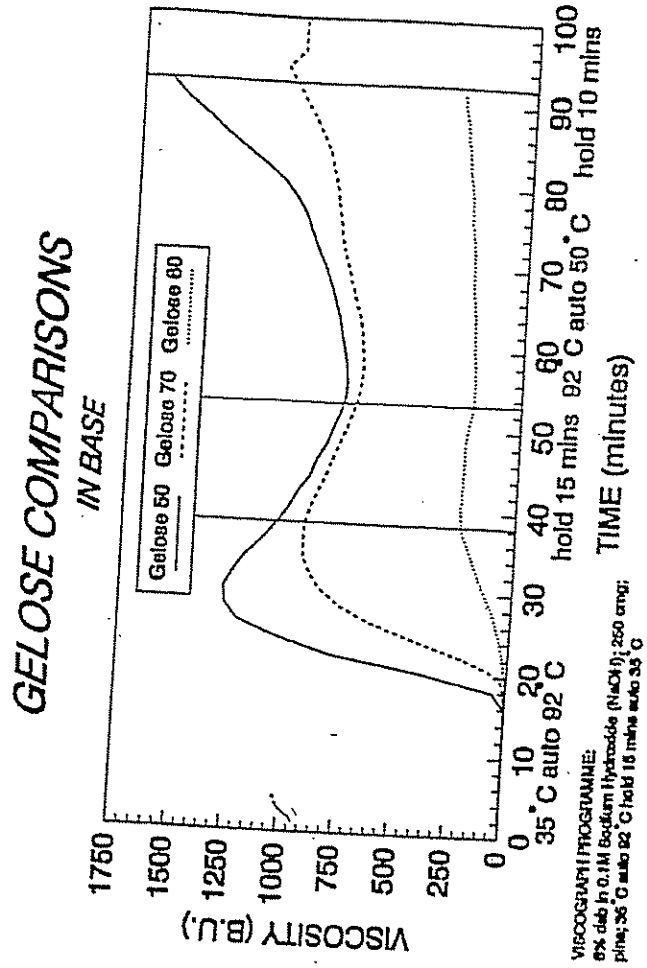


FIG. 3

Total Dietary Fibre Content of High Amylose Maize Starch Fractions
High Amylose 80 (10/91)

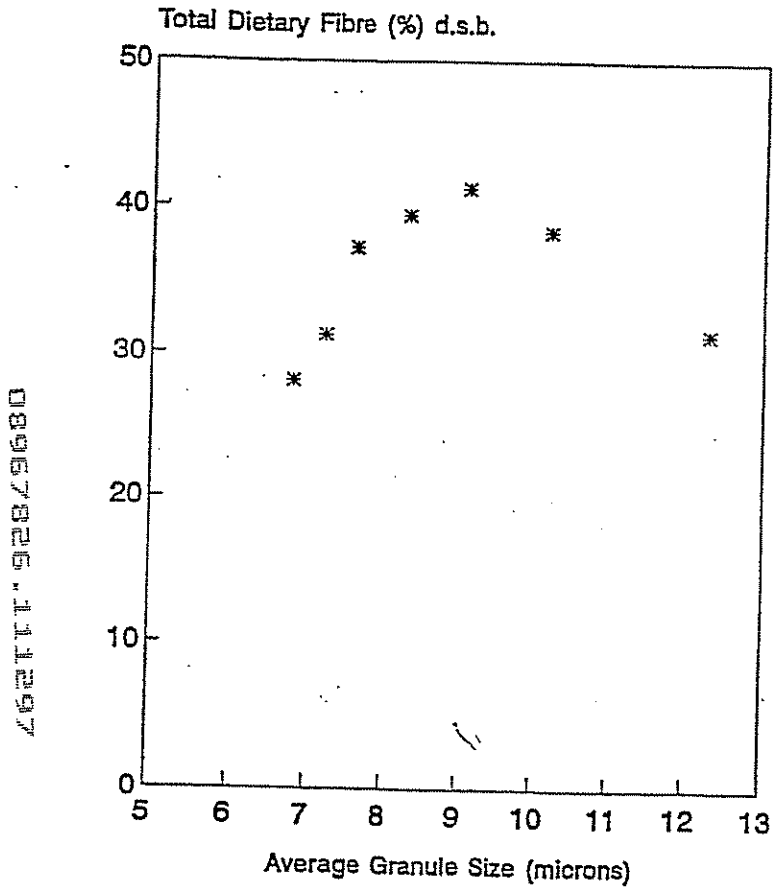


FIG. 4

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1997

Application or Docket Number

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY TYPE ☐ RATE	FEE	OR	OTHER THAN SMALL ENTITY RATE	FEE
BASIC FEE	[redacted]		[redacted]	395.00	OR	[redacted]	790.00
TOTAL CLAIMS	8 minus 20 =	*	x$11=		OR	x$22=	
INDEPENDENT CLAIMS	3 minus 3 =	*	x41=		OR	x82=	
MULTIPLE DEPENDENT CLAIM PRESENT			+135=		OR	+270=	
			TOTAL		OR	TOTAL	790

* If the difference in column 1 is less than zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 5	Minus	** 20	= —
Independent	* 2	Minus	*** 3	= —
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
x$11=		OR	x$22=	
x41=		OR	x82=	/
+135=		OR	+270=	/
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
x$11=		OR	x$22=	
x41=		OR	x82=	
+135=		OR	+270=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

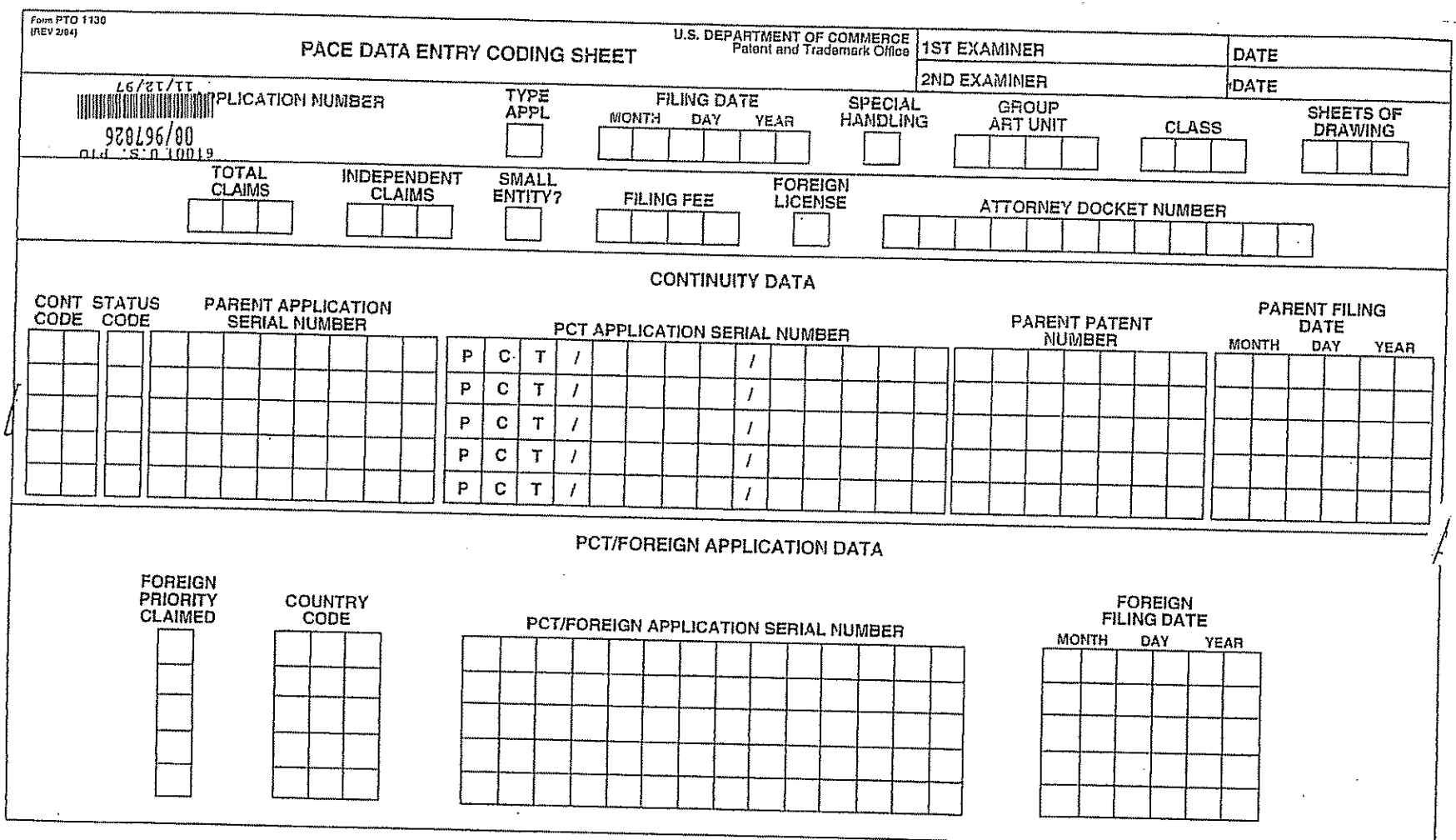
AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
x$11=		OR	x$22=	
x41=		OR	x82=	
+135=		OR	+270=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

FORM PTO-875 (Rev. 8/97) "U.S. Government Printing Office: 1997 - 430-571/63194 Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE



Form PTO 1130
(REV 2/84)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

PACE DATA ENTRY CODING SHEET

1ST EXAMINER	DATE
2ND EXAMINER	DATE

11/12/97
APPLICATION NUMBER
08/967826
61001 U.S. PTO

TYPE APPL

FILING DATE: MONTH DAY YEAR

SPECIAL HANDLING

GROUP ART UNIT

CLASS

SHEETS OF DRAWING

TOTAL CLAIMS

INDEPENDENT CLAIMS

SMALL ENTITY?

FILING FEE

FOREIGN LICENSE

ATTORNEY DOCKET NUMBER

CONTINUITY DATA

CONT CODE	STATUS CODE	PARENT APPLICATION SERIAL NUMBER	PCT APPLICATION SERIAL NUMBER	PARENT PATENT NUMBER	PARENT FILING DATE (MONTH DAY YEAR)
			PCT/ /		
			PCT/ /		
			PCT/ /		
			PCT/ /		
			PCT/ /		

PCT/FOREIGN APPLICATION DATA

FOREIGN PRIORITY CLAIMED	COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FOREIGN FILING DATE (MONTH DAY YEAR)

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] A01H 5/10, C[illegible]/00, A23L 1/308, 1/0522

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A01H 5/10, C08B 30/00, A23L 1/308, 1/0522

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC: AU as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
WPAT KEYWORDS - STARCH, HIGH (W) AMYLOSE, HYBRID, MAIZE, CORN, SEED, C08B
CASM KEYWORDS - HYBRID, MAIZE, CORN, SEED, AMYLOSE, STARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Cereal Chemistry 68(6) 1991 pages 589-596 Szemdrak J. and Pomeranz Y. "Starch and Enzyme-Resistant Starch from High-Amylose Barley". Whole document.	16
A	AU-B-45616/89 (625542) (IOWA STATE UNIVERSITY RESEARCH FOUNDATION INC) 31 May 1990 (31.05.90)	1-15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 October 1993 (01.10.93)	7 OCT 1993 (7.10.93)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929	Authorized officer Mark Donaghey MARK DONAGHEY Telephone No. (06) 2832414

Form PCT/ISA/210 (continuation of first sheet (2)) (July 1992) copljm

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member					
AU 45616/89	EP 372358	HU 896217	HU 63298			
	US 5004864					

END OF ANNEX

F UEST FORM FOR DIVIS NAL APPLICATION UNDER 37 CFR 1.60

#2 Pre-Amdt
C Queen
3/24/98

11/12/97 JC U.S. PTO

DOCKET NUMBER: 1451-007B
Prior Application:
Art Unit: 1804
Examiner: N. Nutter

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

This is a Request for filing a Divisional application under 37 CFR 1.60 of pending prior application Serial No. 08/374,645, filed on April 27, 1995, entitled HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS, by the following named inventor(s):

INVENTOR	RESIDENCE	CITIZENSHIP	POST OFFICE ADDRESS
Kenneth J. McNAUGHT	North Epping, Australia	Australian	18 Marcella Street, North Epping, NSW 2121 Australia
Eric MOLONEY	Tamworth, Australia	Australian	169 Brisbane Street, Tamworth, NSW 2340, Australia
Ian BROWN	Tamworth, Australia	Australian	2 Melissa Avenue, Tamworth, NSW 2340, Australia
Adrian Timothy KNIGHT	Lane Cove, Australia	Australian	18 Nundah Street, Lane Cove, NSW 2066, Australia

1. [X] I hereby verify, to the best of my knowledge, that the enclosed copy of this prior application is a true copy of the above-identified prior application, including the oath or declaration as originally filed.

2. [X] Preliminary Amendment is enclosed.

2a. [X] An Information Disclosure Statement and PTO1449 Form are submitted herewith.

3. [X] Cancel claims 1-7 and 16-23.

4. The filing fee is calculated on the basis of the claims existing in the prior appl. as amended at 2 and 3 above:

	NO. OF CLAIMS		EXTRA CLAIMS	RATE	FEE
Total Claims	8	MINUS 20	0	x $22 =	$0.00
Independent Claims	2	MINUS 3	0	x $82 =	0.00
				Basic Application Fee	790.00
				If multiple dependent claims are presented, add $270.00	
				Total Application Fee	790.00
				Subtract 1/2 if small entity	
				TOTAL APPLICATION FEE DUE	$790.00
				AMOUNT TO BE CHARGED TO DEPOSIT ACCOUNT NO. 12-2237	$790.00

4a. [ ] Enclosed is a Verified Statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27.

4b. [ ] A verified Statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 was filed in prior application and such status is still proper and desired.

5. [X] The Commissioner is hereby authorized to charge fees under 37 CFR 1.16 and 1.17 which may be required, including any extension of time fees to maintain the pendency of the parent application Serial No. 08/374,645 or credit any overpayment to Deposit Account No. 12-2237.

6. [X] Amend the specification by inserting before the first line the sentence:

This application is a division of Application Serial No. 08/374,645 filed April 27, 1995 which is a 371 of PCT/AU93/00389 filed July 30, 1993.

7. [X] Priority of Application Serial No. PL 3894 filed on July 31, 1992, in Japan and Application Serial No. PL 7266 filed on February 12, 1993, in Japan are claimed under 35 USC 119. The certified priority document(s) were acknowledged in Serial No. 08/374,645 on May 22, 1995.

8. [X] The prior application is assigned of record to
Goodman Fiedler Limited
New South Wales, Australia

9. [X] The power of attorney in the prior application is to:

LOWE PRICE LEBLANC & BECKER

10. [X] Also enclosed:

4 pages of formal drawings.

11. [ ] A petition, fee and response has been filed to extend the term in the pending prior application until .

Address all future communications to: (May only be completed by applicant, or attorney or agent of record)

LOWE, PRICE, LEBLANC & BECKER
99 Canal Center Plaza, Suite 300
Alexandria, Virginia 22314

Respectfully submitted,

LOWE, PRICE, LEBLANC & BECKER

Michael G. Gilman
Registration No. 19,114

99 Canal Center Plaza, Suite 300
Alexandria, Virginia 22314
(703) 684-1111 MGG:amz
Date: November 12, 1997
Facsimile: 703-684-1124

#3/Prelim
3
CJ

Docket No.: 1451-007B

PATENT 3/24/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :
:
Kenneth J. McNAUGHT et al. :
:
Rule 60 Divisional of :
Serial No. 08/374,645 : Group Art Unit:
:
Filed: November 12, 1997 : Examiner:
:
For: HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D. C. 20231

Sir:

Prior to examination of the above-referenced application, please amend the application as follows:

IN THE ABSTRACT:

Please insert the enclosed new page 21 to provide an Abstract of Disclosure for the application.

IN THE CLAIMS:

Please cancel claim 8 and replace with the following new claim 24.

24. A maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically

B9

or chemically modified derivatives of maize starch having an amylose content of more than 80%, destructurized maize starch having an amylose content of more than 80%, and non-destructurized maize starch having an amylose content of more than 80%.--

B3 cont'd

Claim 9, line 1, change "8" to --24--.

Please cancel claim 12 and replace with the following new claim 25.

--25. A composition comprising a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives of maize starch having an amylose content of more than 80%, destructurized maize starch having an amylose content of more than 80%, and non-destructurized maize starch having an amylose content of more than 80%.--

B3

Claim 13, line 1, change "12" to --25--.

REMARKS

Entry of this Preliminary Amendment is respectfully requested. Applicants note that claim 8 is rewritten as new claim 24 and claim 12 is rewritten as new claim 25. It is requested that the Examiner renumber the claims in the application to incorporate the changes from this amendment.

Rule 60 Divisional of
Serial No. 08/374,645

In parent application Serial No. 08/374,645, it was determined that an Abstract be presented for the application. Accordingly, presented herewith on separate page 21, is an Abstract for this application.

Please charge any shortage in fees due in connection with the filing of this paper to Deposit Account 12-2237 and please credit any excess fees to such deposit account.

Respectfully submitted,

LOWE, PRICE, LEBLANC & BECKER

Michael G. Gilman
Registration No. 19,114

99 Canal Center Plaza, Suite 300
Alexandria, Virginia 22314
(703) 684-1111 MGG:amz
Date: November 12, 1997
Facsimile: 703-684-1124

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Abstract of the Disclosure

Starch, particularly maize starch, having an amylose content of more than 80% w/w, including physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof. Also, disclosed are hybrid maize seeds capable of producing a starch having an amylose content of more than 80%. Also disclosed are starch fractions of enhanced dietary fiber and/or resistant starch content.

B1